Mycotic aneurysm caused by *Burkholderia pseudomallei*: report of a Brazilian strain genetically related to Thai strains

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Abstract

Melioidosis, a severe infectious disease caused by *Burkholderia pseudomallei* that is prevalent in Southeast Asia and Northern Australia, has been sporadically reported in Brazil since 2003. We report a case of aortic aneurysm with blood culture positive for *B. pseudomallei*. The phylogenetic analysis of 16S ribosomal DNA showed this isolate to be evolutionarily grouped with the MSHR346 strains from Thailand.

Keywords: Aneurysm, Brazil, *Burkholderia pseudomallei*, melioidosis

Original Submission: 22 June 2010; **Revised Submission:** 23 September 2010; **Accepted:** 5 October 2010
Editor: D. Raoult
Article published online: 15 October 2010
Clin Microbiol Infect 2011; **17:** 719–721
10.1111/j.1469-0691.2010.03405.x

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Mycotic aneurysm is a localized, irreversible dilatation of an artery caused by the destruction of the vessel wall by infection. Most mycotic aneurysms are caused by bacteria, despite the name, which was coined by Osler to denote their appearance, which is like 'fresh fungus vegetations' [1]. *Staphylococcus aureus* and serotypes of *Salmonella* non-typhi are the principal micro-organisms involved in cases of mycotic aneurysm [2].

The most frequent aetiology of infected aortic aneurysm appears to be determined by regional conditions and varies according to a region's endemic diseases. In a study of infected aortic aneurysms (80% in men, with average age of 63 years) in an endemic area for melioidosis, the most common comorbidities were hypertension and renal disease. *Burkholderia pseudomallei* was the most commonly isolated pathogen (42.5%) and the abdominal aorta was the most frequently involved artery (87.5%), followed by the thoracic aorta, common iliac arteries and superficial femoral arteries [3].

The first description of mycotic aneurysm caused by *B. pseudomallei* was published in 1998, from a case of a 70-year-old man with hypertension admitted to Veterans General Hospital–Kaohsiung, Taiwan, on 25 November 1994 [4]. Since then, *B. pseudomallei* has been reported as an important pathogen in patients suffering from mycotic aneurysm in endemic regions of Southeast Asia, especially Taiwan, Singapore and Malaysia [5].

Until 2003, melioidosis was generally considered to be restricted to the region between latitudes 20° North and 20° South. However, in 2003 the first cases were observed outside this region, in Northeastern Brazil, during a micro-epidemic in the municipality of Tejuçuoca (3°59′20″ S 39°34′50″ W) in the state of Ceará. Since then, isolated cases and micro-epidemics have been described in various regions of the state [6].

Here we report a case of aortal aneurysm in a male patient from Ceará state, with blood culture positive for *B. pseudomallei*.

On 17 November 2008, a 70-year-old man (a farmer) from the town of Granja, Ceará (3°07′13″ S, 40°49′34″ W), was admitted to an emergency hospital in the region, suffering from intestinal obstruction. The same day an exploratory laparotomy was performed, revealing blood in the abdominal cavity and aortal aneurysm with blood clots in the aneurysmal sac. A standard surgical treatment for abdominal aortic aneurysm that involves replacing the damaged portion of the aorta with an artificial graft was performed. The blood lost was replaced and the patient left the surgical room in a stable condition.

After the laparotomy, antimicrobial therapy was started with sodium cefazolin (1.0 g; intravenously every 8 h), but the patient's condition deteriorated, with surgical wound dehiscence, digestive haemorrhage and sepsis. The antimicrobial therapy was changed to cefepime (2 g; intravenously every 12 h) on 22 November.

On 27 November, the patient was transferred to a general hospital run by the state health service located in the capital, Fortaleza, with worsening of clinical conditions indi-

cating the need for intensive care. He was placed on an artificial respirator. The next day he presented mental disorientation and metabolic acidosis. Blood was then drawn for culture. On 29 November the patient's condition deteriorated further, with arterial hypotension and bradycardia, and vasopressors were administered. At this time, the antimicrobial therapy was changed to piperacillin/tazobactam (4.0 g; intravenously every 8 h). The next day his clinical condition worsened, with evidence of septic shock and renal failure. He died of multiple organ failure.

The result of the blood culture only came back 2 days after the patient's death, revealing the presence of *B. pseudomallei*, identified by VITEK 2™ system (biomérieux, Inc., Hazelwond, MO, USA) and PCR. Additionally, phylogenetic analysis was performed of the 16S region of the nuclear ribosomal DNA and the evolutionary history was inferred using the maximum parsimony method [7]. The bootstrap consensus tree inferred from 100 replicates is taken to represent the evolutionary history of the taxa analysed [8]. Branches corresponding to partitions reproduced in <50% bootstrap replicates are collapsed. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) are shown next to the branches [8]. The maximum parsimony tree was obtained using the Close-Neighbour-Interchange algorithm [9] with search level 3 [8,9] in which the initial trees were obtained with the random addition of sequences (ten replicates). The codon positions included were 1st + 2nd + 3rd + Non-coding. All positions containing gaps and missing data were eliminated from the dataset (Complete Deletion option). There were a total of 1371 positions in the final dataset, out of which one was parsimony informative. Phylogenetic analyses were conducted in MEGA4 [10].

Melioidosis is a severe infectious tropical disease associated with high mortality (septicaemic form), which is prevalent in Southeastern Asia and Northern Australia. In Brazil, the disease and its aetiological agent—an aerobic Gram-negative bacillus, *B. pseudomallei*—have been sporadically reported since 2003 in the Northeast, especially in the state of Ceará [6]. It is normally transmitted through skin wounds and contact with soil and stagnant water. *Burkholderia pseudomallei* is an important pathogen in humans and in a wide variety of animal species in endemic areas, including horses, sheep, cattle, goats, pigs, cats and dogs [11].

Like many other infectious diseases, melioidosis is also known for its diverse clinical manifestations, and for many years in Asia it has been labelled 'the great mimicker' in both the medical literature and the popular press. The most common clinical manifestations include acute, localized, suppurative skin infection, pulmonary infection and septicaemia [12]. However, melioidosis appearing as a mycotic aortic aneurysm, as described here, has rarely been reported [13].

The aortic aneurysm reported here was diagnosed by means of an exploratory laparotomy performed at the time the patient was first hospitalized. Despite the clinical–epidemiological evidence (farmer with fever and abdominal distension, residing in an area where melioidosis had been reported) pointing to melioidosis, with manifestation of mycotic aneurysm, the involvement of *B. pseudomallei* was not considered. After the surgical correction of the abdominal aortic aneurysm, if the hypothesis of mycotic aneurysm caused by *B. pseudomallei* had been considered, the institution of an empirical antimicrobial therapy would have been a better treatment regimen. The microbiological diagnosis was only obtained after the patient's death, because blood cultures were performed late in the illness.

Diagnosis of mycotic aneurysm is difficult because its incidence is low, even in areas where it is endemic, and the clinical presentation is diverse and may be non-specific. It has been suggested that diagnosis can be facilitated if it is considered for febrile patients >40 years of age who reside in or have ever visited areas where *B. pseudomallei* is endemic and who have the following features: (1) abdominal, back or, more rarely, thigh or chest pain; (2) vertebral, paravertebral or retroperitoneal collections; (3) confirmed arterial aneurysm; (4) leucocytosis, or (5) no cause identified after initial investigations [14,15]. The other important clinical scenario in which the diagnosis should be considered is when results of blood cultures are recurrently or persistently positive for *B. pseudomallei* and no other cause has been identified. In this situation, computed tomography scanning of the abdomen with intravenous contrast should be performed, if it is available. Positive blood culture together with a radiological abnormality of the artery involved (usually on computed tomography imaging) strongly suggests bacterial arteritis [16].

Mycotic aortic aneurysm caused by melioidosis remains a difficult disease to manage. Morbidity and mortality are high despite correct diagnosis and aggressive surgical and medical management. It has been proposed that, in endemic areas, the diagnosis of melioidosis should always be considered for persons with appropriate risk factors suffering from mycotic aneurysm, so that suitable antibiotic therapy can be initiated as early as possible before surgical intervention. A course of intravenous ceftazidime with a minimum duration of 4–6 weeks should be considered, and maintenance therapy should include a regimen of two drugs (tromethoprim-sulfamethoxazole and doxycycline), to reduce the risk of relapse [5].

Melioidosis was first described in the state of Ceará only in 2003 and many members of the local medical community are still unaware of its clinical manifestations and so of the

diagnostic and treatment strategies. Because of this, often *B. pseudomallei* infection is not suspected and complementary diagnostic tests are not sought quickly enough, hampering the administration of antimicrobial therapy.

It has been reported that early diagnosis and aggressive management of patients with bacterial arteritis is necessary to ensure better outcome. In endemic countries, *B. pseudomallei* must always be considered as a possible causative agent and high-dose intravenous ceftazidime should be started as soon as possible while awaiting further bacteriological confirmation. Response to treatment is often slow, and the disease is prone to relapse even after years of apparent quiescence. The antibiotic of choice for acutely septicaemic patients is ceftazidime, which is superior to other third-generation cephalosporins and to a combination of chloramphenicol, doxycycline and trimethoprim/sulfamethoxazole [16]. Ceftazidime therapy is equivalent to therapy with imipenem and intravenous amoxicillin/clavulanate with respect to mortality rate, but a higher treatment failure rate is associated with intravenous amoxicillin/clavulanate [17].

The phylogenetic analysis showed that the isolate of *B. pseudomallei* from blood was evolutionarily grouped with the MSHR346, 1106a and 1710b strains of *B. pseudomallei*, all originating from Thailand, and was distant from the *B. pseudomallei* 668 strains originating from Australia (Fig. 1).

Despite the few reports of aortal aneurysms caused by *B. pseudomallei*, in the majority of cases these have occurred in patients contaminated in endemic regions of Southeast Asia. Paradoxically, although Australia is also an endemic zone for melioidosis, no cases of mycotic aneurysm have yet been reported in this country. Nevertheless, it is known that strains of *B. pseudomallei* isolated in the northeast region of Brazil demonstrate homology of the 16S ribosomal DNA with Thai strains, which differ from those from Australia. Our analysis of the 16S ribosomal DNA of the strain from this patient showed that it is phylogenetically closer to the Thai strains. Perhaps this homology of strains is related to the capacity to produce clinical manifestations that are more similar to those found in Southeast Asia.

In closing, it is important to stress that in cases of mycotic aneurysm in patients from regions where melioidosis is endemic, particularly Southeast Asia and Northeast Brazil, the involvement of *B. pseudomallei* should be investigated promptly.

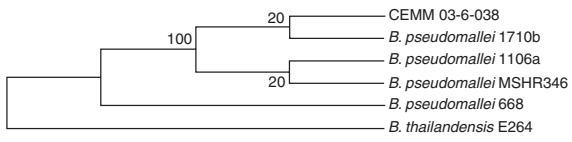

FIG. 1. Evolutionary relationships between *B. pseudomallei* strain CEMM 03-6-038 recovered from a case of mycotic aneurysm in Brazil and strains from Thailand and Australia.

Acknowledgements

The authors are grateful for the financial support of the Brazilian Federal Agency for the Support and Evaluation of Graduate Education (CAPES/PNPD/PRODOC).

Transparency Declaration

The authors declare no conflicts of interest.

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